APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1982, p. 1216–1218
0099-2240/82/051216-03$02.00/0
Vol. 43, No. 5

Microbial Production of 2,3-Butylene Glycol from Cheese Whey

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Received 20 November 1981/Accepted 20 January 1982

Six microorganisms that produced acetoin or diacetyl or both from glucose were tested for the production of 2,3-butylene glycol from lactose. *Bacillus polymyxa* and *Streptococcus faecalis* gave positive results and were tested in unmodified wheys. Cottage cheese whey was unsatisfactory, but *B. polymyxa* produced large amounts of the glycol in sweet whey, about 60 mmol of glycol per 100 mmol of lactose utilized. Aeration and an increased ratio of surface area to volume of whey enhanced the production of glycol. 2,3-Butylene was separated from the spent whey and from acetoin and diacetyl with a Sephadex G-10 column.

About 35 billion pounds of whey are produced annually as a by-product of making cheese in the United States (6). Disposal of the whey is a major problem because whey contains about 5% lactose, and many microorganisms cannot degrade lactose. Research on the utilization of whey has been intensified, but about 40% of the whey produced still is dumped into waterways and sewage systems (6). The purpose of this study was to find microorganisms that can grow in whey, preferably without modification, and convert the lactose to 2,3-butylene glycol, an end product that would be commercially useful. 2,3-Butylene glycol can be converted to 1,3-butadiene (7), the monomer currently supplied by the petrochemical industry, that is polymerized into synthetic rubber. The glycol also is important in making pharmaceuticals, cosmetics, and plastics, and there have been studies attempting to use microorganisms for the production of 2,3-butylene glycol from substrates, with glucose as the energy source (7).

Bacillus polymyxa ATCC 1232, *Enterobacter aerogenes*, *Streptococcus faecalis*, and *Serratia marcescens* (from the culture collection of E. B. Collins) and *Streptococcus diacetilactis* 18-16 (from W. E. Sandine, Oregon State University, Corvallis, Oreg.) were maintained in litmus milk. *Torulopsis colliculosa* NRRL 172 (from the Northern Regional Research Laboratory, U.S. Drug Administration, Peoria, Ill.) was maintained on malt agar slants. All cultures were transferred once per week. Litmus milk, cottage cheese whey, and sweet whey were autoclaved for 13 min at 121°C. Other media were autoclaved for 15 min at 121°C.

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Measurements of pH were made with an Orion model 601 pH meter. Spectrophotometric measurements were made with a Beckman model DB spectrophotometer. Acetoin and diacetyl were determined (usually as the sum of the two) by the Westerfeld procedure (12). 2,3-Butylene glycol was determined by the method of Desnuelle and Naudet (4). Lactose was determined by the Munson-Walker method (3). Acetoin, diacetyl, and 2,3-butylene glycol were separated, using a Sephadex G-10 column, with 0.05 M phosphate buffer (pH 7.2) as the eluant. Effluents during the separations were collected and distributed into test tubes with a Redi-Rak fraction collector (Redi-Rak; LKB Instruments, Inc., Los Angeles, Calif.). When not in use, the glass column (3 cm by 36 cm) was filled with phosphate buffer containing 0.2% sodium azide.

Diacetyl and acetoin were from Eastman Organic Chemicals, Rochester, N.Y. 2,3-Butylene glycol was from K & K Laboratories, Inc., Jamaica, N.Y. Purification and preparation of stock solutions of these compounds were according to previously published procedures (11). Piperazine hexahydrate was from K & K Laboratories, Inc. Creatine was from Calbiochem, La Jolla, Calif., and α-naphthol was from Fisher Scientific Co., Fairlawn, N.Y. Sephadex G-10 column support material and blue dextran 2000 were from Van Waters and Rogers, San Francisco, Calif. All inorganic reagents were reagent grade.

Each of the six organisms was inoculated (1.0%) into Voges-Proskauer (VP) broth, grown for 24 h at 30°C, and tested for VP-positive material (acetoin or diacetyl or both). All were positive, which suggested that the organisms might produce 2,3-butylene glycol. Nevertheless, with lactose substituted for glucose, *Serra-*

TABLE 1. Production of 2,3-butylene glycol by selected microorganisms in unmodified sweet whey (pH 6.0)

Organism	(mmol Glycol production/100 mmol of lactose utilized)[a]			
	3 days		7 days	
	25°C	30°C	25°C	30°C
Bacillus polymyxa	18.1	24.2	43.1	61.1
Torulopsis colliculosa and *Streptococcus diacetilactis*	12.3	16.2	25.1	34.2
Streptococcus diacetilactis	4.8	11.5	6.3	16.2
Torulopsis colliculosa[b]	4.5	6.7	6.1	9.1
Streptococcus faecalis	0.9	2.3	1.1	2.5

[a] The whey contained 135 mmol of lactose per liter; *B. polymyxa* utilized 64.8 mmol per liter (48%) during 7 days at 30°C.

[b] Calculations were based on the assumption that whey contained 0.1% glucose (10) and that all of the glucose (and no lactose) was utilized.

tia marcescens, *E. aerogenes*, and *T. colliculosa* were negative. Since the VP test does not detect 2,3-butylene glycol and *Serratia marcescens* had produced copious amounts of VP-positive material when grown on glucose, spent medium of this organism grown on glucose was tested for glycol. There was none. *Streptococcus diacetilactis* grown on lactose in the broth produced no glycol, but tests were positive for *B. polymyxa* and *Streptococcus faecalis*.

The two organisms that had produced 2,3-butylene glycol in VP broth with lactose substituted for glucose were tested for production of the glycol in autoclaved cottage cheese whey (pH 4.3). We also tested the possibility that *T. colliculosa*, which had produced considerable glycol when grown on glucose, might produce glycol when grown together with *Streptococcus diacetilactis* in the whey. Flasks of whey were inoculated (1%) with the organisms, incubated at 32°C, and sampled at 4-h intervals for 48 h. Titratable acidities, pH values, and plate counts were determined, and the samples were tested for acetoin plus diacetyl and for 2,3-butylene glycol. Plate counts showed that only *Streptococcus diacetilactis* grew, and other tests indicated metabolic activity for only *Streptococcus diacetilactis*. None of the organisms produced 2,3-butylene glycol; *Streptococcus diacetilactis* produced small amounts of acetoin plus diacetyl (ca. 5 μg/ml). Neutralization of the cottage cheese whey most likely would have improved it as a growth medium, but it also might be necessary to make other modifications.

Sweet whey (pH 6.0) was obtained from a cheese factory, placed in 500-ml Erlenmeyer flasks (200 ml per flask), autoclaved, cooled, inoculated (1%) with four selected microorganisms (separately), and incubated (one set of flasks at 25°C and one set at 30°C). One pair of flasks received *Streptococcus diacetilactis* and *T. colliculosa*. Samples, taken each 4 h for 48 h and subsequently at 3, 4, and 7 days, were tested for 2,3-butylene glycol and for acetoin plus diacetyl. Each of the organisms produced 2,3-butylene glycol, especially at 30°C, and especially *B. polymyxa* (Table 1). The bacillus also produced acetoin plus diacetyl (ca. 2 mmol/100 mmol of lactose utilized), which can be readily converted to glycol (5). The mixture of *Streptococcus diacetilactis* and *T. colliculosa* produced more glycol than did either organism growing alone. We assume that the sweet whey contained about 0.1% glucose (10) and that this amount of glucose permitted growth of *T. colliculosa*.

The results of two experiments showed that oxygen influences the production of 2,3-butylene glycol by *B. polymyxa*. In these experiments, the influences of variations in the surface area to volume of growth medium and of aeration were determined. The inoculum was 1% and the incubation time was 72 h at 25°C. In one experiment, two series of three Erlenmeyer flasks (250, 500, and 1,000 ml) containing 100 ml of autoclaved whey per flask were inoculated, incubated, and tested for glycol. In the second experiment, duplicate 500-ml flasks containing 100 ml of autoclaved whey each were inoculated and, during subsequent incubation, one of the two was shaken at 200 rpm with a New Brunswick shaker. With surface area-to-volume ratios of 0.4, 0.8, and 1.2, the amounts of glycol produced per 100 mmol of lactose utilized were 16.5, 19.9, and 26.8 mmol, respectively. With shaking, the organism produced 39.8 mmol of glycol per 100 mmol of lactose utilized; without shaking, it produced only 22.2 mmol/100 mmoles of lactose.

Separation of the glycol produced from glucose in industrial wastes has been reported to be difficult (7, 9), but we found that the glycol easily separated from whey and from acetoin and diacetyl with a Sephadex G-10 column. The results (Fig. 1) are from an experiment in which sweet whey was inoculated with *B. polymyxa* and was

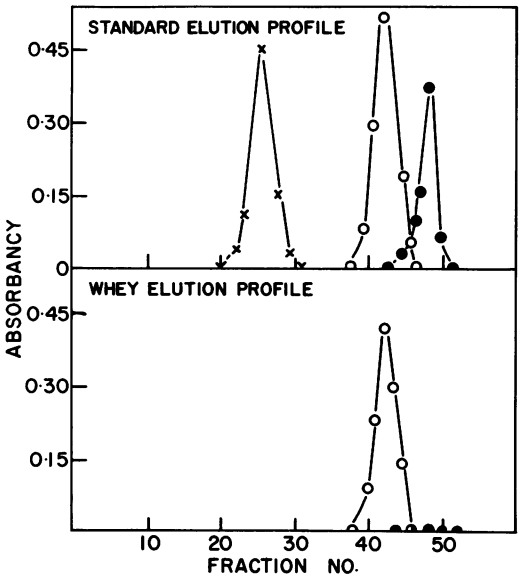

FIG. 1. Separation of 2,3-butylene from acetoin and from whey with a Sephadex G-10 column. Symbols: ○, 2,3-butylene glycol monitored by the method of Desnuelle and Naudet (4); ●, acetoin monitored with the Westerfeld procedure (12); X, blue dextran monitored at 630 nm. Fraction volume, 6.5 ml. The standard elution profile was obtained by separation of a known mixture. The whey elution profile was obtained by separation of 2,3-butylene glycol from whey after production of the glycol in the whey by *B. polymyxa*.

incubated for 24 h at 25°C. Ten milliliters of the spent medium was placed at the top of the column and eluted with phosphate buffer at a flow rate of 1.5 ml/min.

Our studies indicate that it might not be difficult to develop procedures for the commercial production of 2,3-butylene glycol from sweet whey, although unmodified cottage cheese whey was not satisfactory. The optimal pH for the production of glycol from glucose in industrial wastes was 6.0 (8), the exact pH of the sweet whey that we obtained. Some industrial wastes and natural products required the addition of phosphorus (7). Sweet whey apparently contains the growth factors required for adequate growth and production of glycol by *B. polymyxa*. Agitation decreased the production of glycol from glucose (1), and aeration with compressed gases increased the production (2). We found that agitation and an increased ratio of surface area to volume enhanced production of glycol by the bacillus.

The maximal yields we obtained were about 60 mmol of 2,3-butylene glycol and about 2 mmol of acetoin per 100 mmol of lactose. This was a conversion to useful products of over 30% of the lactose utilized. It is possible that with further studies methods can be refined so as to obtain even greater yields from the lactose in sweet whey.

This investigation was supported in part by a grant from the Dairy Council of California.

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